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Published in:
Journal of Cystic Fibrosis

DOI:
10.1016/j.jcf.2015.01.006

Publication date:
2015

Journal of Cystic Fibrosis 14 (2015) 169 – 177

www.elsevier.com/locate/jcf

Review

Cystic fibrosis and the role of gastrointestinal outcome measures in the new era of therapeutic CFTR modulation ☆

Frank A.J.A. Bodewes [a,*], Henkjan J. Verkade [a], Jan A.J.M. Taminiau [b], Drucy Borowitz [c], Michael Wilschanski [d] Working group Cystic Fibrosis and Pancreatic Disease of the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN)

[a] *Pediatric Gastroenterology and Hepatology, University of Groningen, University Medical Center, Groningen, The Netherlands*
[b] *Pediatric Committee, European Medicines Agency, London, United Kingdom*
[c] *Department of Pediatrics, State University of New York at Buffalo School of Medicine and Biomedical Sciences, Women and Children's Hospital of Buffalo, Buffalo, NY, United States*
[d] *Pediatric Gastroenterology, Hadassah Hebrew University Medical Center, Jerusalem, Israel*

Received 29 October 2014; revised 20 January 2015; accepted 20 January 2015
Available online 10 February 2015

Abstract

With the development of new drugs that directly affect CFTR protein function, clinical trials are being designed or initiated for a growing number of patients with cystic fibrosis. The currently available and accepted clinical endpoints, FEV1 and BMI, have limitations.

The aim of this report is to draw attention to the need and the ample possibilities for the development and validation of relevant gastrointestinal clinical endpoints for scientific evaluation of CFTR modulation treatment, particularly in young children and infants.

The gastrointestinal tract offers very good opportunities to measure CFTR protein function and systematically evaluate CF related clinical outcomes based on the principal clinical gastrointestinal manifestations of CF: intestinal pH, intestinal transit time, intestinal bile salt malabsorption, intestinal inflammation, exocrine pancreatic function and intestinal fat malabsorption.

We present a descriptive analysis of a variety of gastrointestinal outcome measures for clinical relevance, reliability, validity, responsiveness to interventions, feasibility in particular in young children and the availability of reference values.

Keywords: Cystic fibrosis; Outcome measures; End points; Gastrointestinal; Clinical trials; Intestinal pH; Intestinal transit time; Bile acid metabolism; Intestinal inflammation; Exocrine pancreatic insufficiency; Fat malabsorption

Contents

☆ The views expressed in this article are those of the authors and do not necessarily reflect official positions or policies of the European Medicines Agency.

* Corresponding author at: Department of Pediatric Gastroenterology and Hepatology, University Medical Center Groningen, PO Box 30001, 9700 RB Groningen, The Netherlands. Tel.: +31 503614147; fax: +31 503646746.
E-mail address: f.a.j.a.bodewes@umcg.nl (F.A.J.A. Bodewes).

http://dx.doi.org/10.1016/j.jcf.2015.01.006
1569-1993/

1. Introduction

In recent years great progress has been achieved in therapeutic Cystic Fibrosis Transmembrane Corrector (CFTR) protein modulation. Current CFTR modulation treatment is based on the use of small molecules, that either improve gating of ions ("CFTR potentiators") or restore folding ("CFTR correctors") of the CFTR protein to improve its function. Clinical trials testing CFTR modulators have proven their success by showing significant clinical improvement, including sustained improvement in lung function as measured by forced expiratory volume in 1 s (FEV1), and an increase in body mass index (BMI) [1,2].

To date the major accepted and established clinical outcome measures for cystic fibrosis (CF) are FEV1, body weight and body mass index (BMI). However, unlike previous symptomatic treatment approaches for cystic fibrosis, therapeutic CFTR modulation offers the prospect of early intervention and possible preemptive treatment. As a consequence, clinical trials to prove efficacy will be performed in increasingly younger cystic fibrosis patients [3]. Young children and infants with cystic fibrosis, in particular those diagnosed via neonatal screening, may have well preserved lung function and normal growth. Therefore, development and validation of clinical outcome measures applicable in this young age group are imperative.

The gastrointestinal tract offers opportunities to measure CFTR protein function and systematically evaluate CF related clinical outcomes for clinical trials. Clinical signs of CFTR dysfunction in the gastrointestinal tract often occur earlier in disease development than in the respiratory tract. Meconium ileus is almost pathognomonic for CF. Meconium ileus will usually be symptomatic before neonatal screening results are available. Exocrine pancreatic insufficiency can present at birth or develop in weeks to months during the first year of life [4]. Additionally the same pathophysiological triad of obstruction, infection, and inflammation that causes disease in the airways also causes disease in the intestine [5].

In this position paper we systematically discuss gastrointestinal outcome measurements for cystic fibrosis that are available to date. We describe clinically measurable outcome parameters and methods to directly measure CFTR protein function in gastrointestinal tissues. The aim of the report is to draw attention to the need and the ample opportunities for the development and validation of relevant clinical endpoints for scientific evaluation of CFTR modulation treatment, particularly in young children and infants.

2. Methods

After an expert group assessment we systematically discuss potential high-impact gastrointestinal outcome measurements based on a retrospective literature analysis. We structured our search for potential GI outcome measures on the principal clinical gastrointestinal manifestations in CF: intestinal pH, intestinal transit time, intestinal bile salt malabsorption, intestinal inflammation, exocrine pancreatic function and intestinal fat malabsorption. We present a descriptive analysis of gastrointestinal outcome measures for clinical or disease relevance, reliability, validity, responsiveness to interventions, feasibility in particular in young children and the availability of reference values. The results are summarized in the Table 1.

3. Results

3.1. Measurements of clinical gastrointestinal manifestations of cystic fibrosis

3.1.1. Intestinal pH profile

Physiologically, gastric acid is buffered by secretion of bicarbonate by the pancreas and by the enterocytes of the proximal small intestine. CFTR is essential for adequate pancreatic and duodenal bicarbonate secretion. In patients with CF, the pancreatic and duodenal bicarbonate secretion is insufficient to neutralize the gastric acid load [6,7]. Hence, the duodenal pH is (on average) 1–2 units lower in CF patients compared with healthy controls. Accordingly, CF patients have significantly longer postprandial periods in which the duodenal pH is below 4 [8]. In the proximal intestine, acidification may interfere with absorption both by inhibiting pancreatic enzyme activity [9] and by causing intraluminal precipitation of bile acids with impaired mixed micelle formation [10]. More distally in the small intestine, the pH values of jejunal and ileal contents from CF patients vary from lower to similar pH values compared with healthy controls. Bicarbonate secretion is tightly tied to fluid secretion [11] and in the CF mouse intestine is essential to allow mucins to unfold and become fluid [12,13]. Thus, the excess acidity in the intestine may contribute to obstruction.

Table 1
Schematic representation for the availability of the epidemiological qualities of gastrointestinal outcome measures for cystic fibrosis.

	Test	Outcome measured	CFTR related	Clinical relevance	Reliability	Validity	Reported response	Feasibility young age	Reference values
Downstream evidence of CFTR dysfunction	pH pill	Intestinal pH	+	?	+	+	+	−	+
	Scintigraphy	intestinal motility	+	+	+	?	?	−	+
	Wireless motility capsule	Intestinal motility	+	+	+	+/−	−	−	+
	Plasma C4	Fecal bile salt loss	?	?	+	?	?	+	+/−
	Plasma FGF19	Fecal bile salt loss	?	?	+	?	?	+	+/−
	Fecal calprotectin	Intestinal inflammation	?	?	+	?	?	+	+
	GI endoscopy	Intestinal inflammation	+	?	+	?	−	+/−	+
	Capsule endoscopy	Intestinal inflammation	+	?	+/−	+/−	−	−	+/−
	Fecal elastase-1	Exocrine pancreatic function	+	+	+	+	−	+	+
	CFA	Steatorrhoea	+/−	+	+/−	+	+	+/−	+
	Malabsorption blood test	Steatorrhoea	+/−	+	+/−	+/−	+	?	+
	Free fatty acid absorption test	PERT independent fat malabsorption	?	+	+/−	+/−	−	+	+
Direct measurement of CFTR function	Intestinal current measurement		+	?	+	+	?	+	+
	Intestinal organoid volume		+	?	?	?	+	+	?

+ evidence available.
− no evidence available.
? no information available.
C4: plasma 4-cholesten-3-one.
FGF19: Fibroblast growth factor 19.
CFA: coefficient of fat absorption.
PERT: pancreatic enzyme replacement therapy.

Using a wireless motility and pH detecting intestinal capsule, Gelfond et al. recently showed in CF patients *in vivo* a deficient buffer capacity for neutralization of the gastric acid in the proximal small bowel [14]. In another study using a before-and-after design, adult patients with the G551D CFTR mutation had abnormal buffering capacity at baseline but had near normalization of intestinal pH after taking the CFTR potentiator Ivacaftor [15]. The latter study indicates the potential role of this clinical tool in clinical trials. Due to the size of the device, the pH capsule has limitations for use in children and infants. The correlation between improved buffering and fat absorption or body weight improvement is not yet available.

3.1.2. Intestinal transit time measurements

Different studies have revealed a prolonged intestinal transit time in adult and pediatric cystic fibrosis patients with exocrine pancreatic insufficiency compared to healthy controls [16–18]. The prolonged intestinal transit time is not corrected by pancreatic enzyme replacement therapy. Possible factors that may be involved in the prolonged bowel transit time in patients with CF include the "ileal brake" seen with fat malabsorption, small bowel bacterial overgrowth, distal intestinal obstruction syndrome (DIOS), dysmotility disorders (constipation) and a history of intestinal surgery [17]. In older studies the oro-cecal transit time (OCTT) using lactulose/hydrogen breath test has been used to determine small intestinal transit [8]. It revealed that most cystic fibrosis patients have a measurable prolonged OCTT of at least +50% in the fasted state [19,20]. While breath testing is easy to perform, there are limitations that complicate interpretation and can affect the sensitivity and specificity of this approach [21].

3.1.2.1. Intestinal transit time scintigraphy. When using scintigraphy to measure intestinal transit time, subjects ingest an orally administered radioactive tracer and then are monitored for the distribution of the tracer within the stomach and small intestine [22]. Nuclear scanning images are obtained at regular intervals. The percentage arrival of total small bowel activity at the terminal ileum and cecum/ascending colon at 6 h can be used as an index of small intestinal transit time. Intestinal transit time scintigraphy is considered to be less variable compared with indirect methods like breath tests. However, the need for frequent repeated scans makes the method time-intensive. There is a significant intra-subject biological variability (~20%) for small bowel transit times [23]. As a consequence the test is only discriminative in the extreme ranges. Scintigraphy has been used in clinical trials for intestinal dysmotility and IBS [24,25]. The need for use of a radioactive tracer makes the method less suitable for routine use in clinical trials or for use in the pediatric age group.

3.1.2.2. Intestinal transit time wireless motility capsule. A new option to determine intestinal transit time is the wireless motility capsule (WMC) System. The WMC can be swallowed by the subject. Subsequently the transit time through the small intestine can be determined based on the pH landmarks of stomach, intestine and colon. The WMC method correlates well with the scintigraphy method [26]. The WMC does not impose

the hazard of radioactive markers and can be performed in an ambulatory setting. For determining small intestinal transit time with the WMC method a sensitivity of 0.42 and a specificity of 0.95 has been reported in patients with constipation when compared to radiopaque marker studies [27]. The intestinal transit time wireless capsule has been used to determine the small bowel transit time in CF patients [28]. It revealed significant and reproducible delay in the small intestinal transit of CF patients compared to normal controls. The use of WMC is limited in young children and infants due to its size (26 mm × 13 mm) and lack of regulatory approval for use in children. However, small bowel video capsule endoscopy devices of similar size (26 mm × 11 mm) have been approved for use in children down to age 2 years (see below).

3.1.3. Intestinal bile salt malabsorption

A deregulated bile salt homeostasis including increased fecal bile salt excretion is an intrinsic and consistent phenotype of cystic fibrosis and presumably directly related to decreased CFTR protein function. The increased fecal bile salt excretion is independent from CF related intestinal fat malabsorption or exocrine pancreatic insufficiency [29]. The measurement of the total bile salt content in feces over a given period of time is considered the gold-standard for diagnosing intestinal bile salt loss. Stool should be collected for at least 48 h or longer to overcome variation in excretion [30].

Intestinal bile salt malabsorption can also be diagnosed with the use of radioactive ^{75}Se-labeled homocholic acid-taurine (*^{75}SeHCAT)* [31]. Using the SeHCAT method O'Brien et al. have reported a three-fold higher fecal bile malabsorption in CF patients compared to healthy controls that was not related to fecal fat excretion [32]. Although proven to provide significant and relevant information for the diagnosis of intestinal bile salt loss, SeHCAT scan is not suitable for wide scale use in clinical trials because of the need for the use radioactive tracers. New relevant plasma markers for bile salt synthesis and intestinal bile salt absorption have recently become available and are discussed below.

3.1.3.1. Plasma 4-cholesten-3-one (C4). Plasma 4-cholesten-3-one (C4) is an intermediate product of the bile salt biosynthesis and a marker of CYP7A1 enzyme activity [33]. CYP7A1 is the rate-limiting enzyme in the bile salt synthesis in the liver. Thus, C4 can be used as a marker for hepatic bile salt synthesis. In patients with increased intestinal bile salt loss, the plasma C4 levels are increased compared to normal controls as a marker of the compensatory increase in hepatic bile salt synthesis. Thus, C4 can function as a surrogate marker for intestinal bile salt loss. Perturbations in C4 levels have been reported in several clinical studies concerning irritable bowel syndrome, inflammatory bowel disease and cholestatic liver diseases, however to date not in cystic fibrosis [34,35]. In patients with bile acid malabsorption (BAM), either after ileocecal resection surgery (BAM type 1) or with the idiopathic form of intestinal bile salt malabsorption (BAM type 2), plasma 4-cholesten-3-one had a sensitivity/specificity of respectively 90%/77% and 97%/74%, respectively, to diagnose the intestinal bile salt malabsorption [36]. C4 measurement can be performed in young children and infants [37]. C4 has been proven to respond to interventional treatments in patients with an increased fecal bile salt loss [33,38].

3.1.3.2. Plasma fibroblast growth factor 19 (FGF19). FGF 19 is an intestine-derived signaling protein that functions as an enterohepatic hormone, regulating hepatic bile acid synthesis and carbohydrate metabolism. Plasma FGF19 concentration is a marker of intestinal bile salt re-absorption. In patients with increased intestinal bile salt loss the FGF19 plasma levels are decreased as a sign of the disrupted enterohepatic circulation [39]. In patients with bile salt diarrhea it was reported that FGF19 was positively related to SeHCAT retention. In non-CF patients with severe fecal bile salt loss, FGF19 had a sensitivity/specificity of respectively 67%/77% to diagnose intestinal bile salt loss [40]. In this same study, Pattni et al. found that FGF19 could reliably predict the clinical response of patients on bile salt sequestration therapy. Together, these observations indicate that FGF19 levels can be used for diagnosis in conditions of intestinal bile loss, in particular also for the detection and quantification of bile salt re-absorption in CF. FGF19 measurement can be performed in young children and infants [41].

3.1.4. Intestinal inflammation

Several studies have shown evidence of intestinal inflammation in cystic fibrosis, particularly in patients with pancreatic insufficiency. The underlying pathophysiology of intestinal inflammation is not completely understood. Data from cystic fibrosis mouse models suggest that the intestinal inflammation is related to CFTR dysfunction in the intestinal tissues, which leads to mucus accumulation, disturbed motility, small bowel bacterial overgrowth and inflammation with altered innate immune responses. It is to be expected that these different factors interact with one another [42].

3.1.4.1. Fecal calprotectin. To date several fecal inflammatory biomarkers are available of which fecal calprotectin is the most studied and used in the clinical practice [43,44]. Calprotectin is a very stable protein originating from inflammatory neutrophils. The value of fecal calprotectin in the diagnosis of intestinal inflammation has been established for inflammatory bowel disease (IBD). A sensitivity and specificity for fecal calprotectin of 89% and 81%, respectively, to diagnose IBD, has been reported in pooled data from adults and children [45]. Additionally, fecal calprotectin has been successfully used to establish therapeutic effects of anti-inflammatory agents in clinical IBD trials [46]. Several studies have demonstrated significantly increased fecal calprotectin levels in cystic fibrosis patients compared to healthy controls [47]. The fecal calprotectin levels do not seem to be related to the presence of small intestinal bacterial overgrowth that is frequently observed in cystic fibrosis patients [48,49]. Clinical trials using probiotics to treat intestinal inflammation in cystic fibrosis patients have shown a therapeutic responsiveness of fecal calprotectin indicating the potential of this marker as a clinical endpoint [50]. Fecal calprotectin is physiologically increased in children under the age of 3 years making this marker less suitable

for this younger age group [51]. Calprotectin is not specific to intestinal inflammation. For example calprotectin is also increased in pulmonary secretions during lung infections. This should be taken into consideration in cystic fibrosis where the combination of pulmonary and intestinal inflammation may coexist.

3.1.4.2. Gastro intestinal endoscopy and intestinal histology. Video-endoscopy can be performed as an upper esophageal-gastric-duodenal or ileocolonic endoscopy. In this manner hallmarks of inflammation can be visualized and scored. Additionally, tissue for histological assessment can be obtained. Histological signs of inflammation can by systematically evaluated using endoscopic biopsy material. Endoscopic evidence for chronic intestinal inflammation in the duodenum of cystic fibrosis patients has been reported [52,53]. Endoscopy is a well-established and validated method for identifying intestinal inflammation and the gold standard to determine therapeutic effects. However, endoscopic procedures are rather invasive diagnostic procedures with the necessity for sedation or general anesthetic making the procedure less applicable for children and infants.

3.1.4.3. Small bowel capsule endoscopy. A relatively new option for making the diagnosis of intestinal inflammation is small bowel capsule endoscopy (SBCE). The primary indication of SBCE is to examine areas of the small intestine that cannot be inspected by other types of endoscopy. One study reported SBCE results in CF patients [49]. The study showed that most cystic fibrosis patients had varying degrees of diffuse areas of inflammatory findings in the small bowel including edema, erythema, mucosal breaks, and frank ulcerations. The method of SBCE has been well established as a descriptive diagnostic tool for intestinal inflammation. Sensitivity and specificity for detecting distal small-bowel Crohn's disease with SBCE has been reported to be 100% and 91%, respectively [54]. However, systematic quantitative evaluation of SBCE is more difficult and has not yet been well established. SBCE has been used as an outcome measure in clinical trials but not yet in therapeutic trials for cystic fibrosis. SBCE was also approved by United States Food and Drug Administration (FDA) for use in children 2 years of age and older [55]. Due to the size of the video capsule SBCE in young children and infants has to be introduced via endoscopy, rather than by (attempted) swallowing.

3.1.5. Measures of exocrine pancreatic function

Exocrine pancreatic insufficiency (EPI) develops in most CF patients with severe CFTR genotypes. EPI is secondary to CFTR related auto-digestion and fibrosis of pancreas in patients with severe CFTR mutations. EPI develops mostly in the first weeks or months of life, however can also develop later in life. The EPI related maldigestion is treated with pancreatic enzyme replacement therapy (PERT). To date established EPI in CF is considered an irreversible disease state. However, it cannot be excluded that new CFTR modulation therapies may be able to improve already developed EPI and the intestinal absorption of nutrients. Support for this possibility can be derived from the ivacaftor trials showing an improved intestinal pH upon treatment, which, at least partly, may be due to improved pancreatic bicarbonate secretion. If EPI could be shown to be completely or partially reversible, this could be interpreted as a measurement of restoration of pancreatic CFTR function. Therefore, it is reasonable to monitor the exocrine pancreatic function in clinical trials with CFTR modulators in particular young children and infants. The most used and widely available exocrine pancreatic function test is the measurement of fecal elastase-1 [56]. Other tests include direct pancreatic function measurement via the secretin-cholecystokinin (CCK) stimulation test and serum immunoreactive trypsinogen (IRT) measurement [57,58].

3.1.5.1. Fecal elastase-1. Fecal elastase-1 is one of the proteolytic pancreatic enzymes. In the intestine elastase-1 is not subject to degradation. It is pH and temperature stabile and is excreted via the feces. A small fecal sample can easily be obtained and stored. Elastase-1 has no cross reactions with chymotrypsin of animal sources that is present in exogenous submitted pancreatic enzymes (PERT). For this reason elastase-1 can still be used a marker of exocrine pancreatic function during PERT use. In the case of exocrine pancreatic insufficiency fecal elastase-1 is decreased. Reference values of fecal elastase-1 have been established [59]. The positive predictive value of a fecal elastase-1 of less than 100 μg/g for detecting pancreatic insufficiency in patients with cystic fibrosis is very good; a negative test (>100 μg/g stool) has 99% predictive value for ruling out EPI [60]. The level of fecal elastase-1 varies with age. Fecal elastase-1 is low in meconium. Normal values are reached within 3 days after birth and in 2 weeks in premature infants independent of gestational age. Hereafter fecal elastase-1 values in children are comparable to adult levels.

3.1.5.2. Direct pancreatic enzyme secrets measurements. The CCK test is considered the gold standard in the evaluation of exocrine pancreatic insufficiency. A tube is passed into the duodenum to enable the collection of secretions for analysis. Secretin and CCK are cholecystokin given intravenously. Duodenal aspiration samples are taken at set intervals for analysis. The CCK test has between reported for use in patients with CF and is capable of reliably differentiating EPI from PS patients [61]. However, the procedure is complex, invasive, expensive and time consuming. Globally, only a few centers have the experience to perform the sampling and enzyme analysis. These drawbacks make this method less suitable as an endpoint measurement method for clinical trials in particular in young children and infants.

3.1.5.3. Serum immunoreactive trypsinogen. Trypsinogen is the precursor protein of trypsin that is synthesized and stored in the pancreas and released into the small intestine. Trypsinogen is also secreted into the blood by the pancreas. A particular isoform (cationic), IRT, can then be measured in serum using radioimmunoassay. IRT can be elevated in conditions like pancreatitis. IRT is decreased compared to normal controls in the situation of complete EPI. However in infants and children with CF who develop EPI over the course of the first years of life a distinct pattern for the IRT has been observed. In the

neonatal period, patients present with distinctly higher than normal IRT values. Over the course of several years the IRT levels decrease gradually below normal values indicating EPI. As a result of this fluctuating pattern in the first decade of life, IRT is not suitable for determining EPI in infants. IRT can, however reliably, determine EPI in children with CF over 7 year of age [57].

3.1.6. Intestinal fat malabsorption

A prominent feature of the gastrointestinal cystic fibrosis phenotype is intestinal fat malabsorption ([62]. Intestinal fat malabsorption in CF is related primarily to two pathological mechanisms. The first is disrupted lipolysis of dietary triglycerides primarily caused by EPI. A decreased bile salt pool and/or precipitation of bile salts can impair micelle formation leading to an impairment of the intestinal, post-lipolytic, absorption of free fatty acids and monoglycerides.

3.1.6.1. Coefficient of fat absorption. The coefficient of fat absorption (CFA) is the current clinical standard for measuring intestinal fat absorption. CFA compares the dietary fat intake to the fecal fat excretion measured over a period of 72 hours [63,64]. CFA has historically often been used as an outcome measure in clinical trials. CFA has been shown to measure therapeutic effects in particular of PERT medication. The CFA methods measure the total fat excreted and do not differentiate between lipolytic or post-lipolytic fat malabsorption. The advantage of the lack of discrimination is that irrespective of the pathophysiological mechanism by which CF affects fat absorption, the clinically relevant result is quantified. In patients with CF, CFA is largely influenced by the use and effectiveness of PERT, although some patients may have moderate to severe steatorrhea as measured by CFA, despite seemingly adequate doses of PERT [65]. To a lesser extend CFA may reflect changes the *intestinal* CFTR protein function. CFA has several drawbacks in both the execution and interpretation. Therefore it can be difficult to achieve research-quality CFA measurements in infants, toddlers and young children [66]. However selected use of carefully-conducted fecal fat balance studies may help clarify whether the weight gain seen with CFTR modulation is as a result of improved fat absorption.

3.1.6.2. Stable isotope free fatty acid absorption test. If a more detailed analysis is warranted to determine whether the fat malabsorption is mainly due to impaired lipolysis or to impaired uptake of fatty acids, other tests are available. The free fatty acid absorption can be quantified using the stable isotope free fatty acid absorption test. In this test intestinal absorption of the non-radioactive ^{13}C-labeled dietary free fatty acid is estimated based on plasma enrichment of the label after oral ingestion.

If required, the stable isotope free fatty acid can be combined with a stable isotope labeled triglyceride. The stable isotope free fatty acid absorption test has been used in CF patients and can be performed in young children and even infants [67,68] In a stable isotope study in CF patients a strong relation was demonstrated between free fatty acid absorption and CFA ($r = 0.88, P < 0.001$) [67]. In term and preterm neonates the stable isotope free fatty acid absorption highly correlated with the efficacy of fat absorption ($r = 0.82$, $p = 0.02$; and $r = 0.91$, $p = 0.004$; respectively) [68]. No reports are available for using the stable isotope free fatty acid test in the setting of CFTR modulating trials.

3.1.6.3. Malabsorption Blood Test. The Malabsorption Blood Test (MBT) is a new method to assess the degree of intestinal fat malabsorption. It is based on the principle of comparing the simultaneous absorption of a free fatty acid (pentadecanoic acid) and a triglyceride (triheptadecanoic acid) requiring hydrolysis before intestinal absorption. These marker lipids can be quantifiably measured in the blood after intestinal absorption as a reflection of intestinal fat absorption. In a pilot study it was demonstrated that in subjects over 12 years of age, the MBT has been shown to respond to changes in fat absorption in healthy subjects using a lipase inhibitor and in subjects with CF while on or off enzyme therapy [69].

Although potentially an absorption blood test offers a practical alternative for the CFA it has to be taken into consideration that plasma measurements of fatty acids levels do not necessary reflect the total intestinal absorptive capacity of dietary lipids [70].

3.2. Direct CFTR function measurement in the intestine

3.2.1. Intestinal electric current measurements (ICM)

Intestinal electrical current measurement (ICM) is an *ex vivo* electrophysiological method in which a freshly obtained rectal biopsy is evaluated for its electrical responses related to chloride ion transport. The procedure is performed by mounting a fresh rectal biopsy in an Ussing chamber and adding a series of chemicals baths stimulating secretion (secretagogues), according to reported protocols [71]. It is a method that assesses functional CFTR activity. There is a clear difference in ICM between normal (non CF) and abnormal (CF) results. Considerable technical expertise is required to place the tissue in the Ussing chamber and add the secretagogues in the correct manner. The potential of ICM was recently extensively reviewed in several reports [72–74]. ICM is a safe method that is used as a diagnostic aid for non-classic cases of CF mainly in infants and young children. ICM changes have not been reported in relation to CFTR modulation.

3.2.2. Intestinal organoid volume measurement (IOVM)

Intestinal organoids are *ex-vivo* stem cell-derived human epithelial 'mini-organs' from tissues of patients that closely resemble the organ structure and function of intestinal mucosa. Intestinal organoids can be derived from rectal biopsy specimens of patients. Rectal biopsies can be obtained safely in newborns and infants. Organoids of individual patients can be stored for later *ex-vivo* use or testing. Intestinal organoid volume measurements have been developed and have been shown to indirectly represent CFTR protein function [75]. Furthermore quantitative changes in organoid volume have been related to changes in therapeutic modulation of CFTR function [76].

4. Discussion

Clinical trials with CFTR modulators are currently being designed and implemented for increasing numbers of patients. The CFTR modulator ivacaftor already has proven to improve lung function and body weight in the older children and adults with the G551D CFTR mutation. However, additional treatment options for other CFTR mutations types and treatments early in disease development are currently pursued. In the future, even preemptive or preventive treatment could be considered.

The gastrointestinal tract offers several unique opportunities to study CFTR protein function and CFTR related clinical outcome measures. Importantly many of the gastrointestinal manifestations of cystic fibrosis are already present at birth or in childhood, making them potentially suitable for studying treatment effects in young children and infants. However, not all GI outcome measures are feasible to perform in small children.

Less emphasis has been given to the development of gastrointestinal outcome measures for therapeutic trials in cystic fibrosis than for pulmonary outcome measures. This was partially due to the presumed irreversible character of CF gastrointestinal manifestations, for example exocrine pancreatic insufficiency. Different from pulmonary disease, some GI manifestations of CF do not present with clinically recognizable and relevant disease. For example, diminished GI bicarbonate secretions or fecal bile salt loss, clear entities of the CF phenotype, do not directly correlate to a distinct clinical pathology. The current studies with CFTR modulators offer new and promising opportunities to implement GI outcome measures for cystic fibrosis, and the use of GI outcome measures can provide evidence of the systemic action of these medications.

Although promising, many of the gastrointestinal outcome measures suggested and discussed in this paper still have to be further developed into valid clinical endpoint for use in clinical trials. To date some gastro-intestinal outcome measures are already evaluated in post marketing evaluations of CFTR modulators. The goal to develop new outcome measures should be a joint ambition of all institutional partners involved in drug development for cystic fibrosis. Academics and CF clinical researchers strive to provide the opportunities and theoretical background for potential new outcome measures. Pharmaceutical companies understand the shared obligation to support outcome measure development. Medicine regulatory bodies, like the U.S. Food and Drug Administration and European Medicines Agency, support and enforce outcome measure development in their advice and legislation. Additionally, CF patient interest groups prioritize and support endpoint development as an important goal for cystic fibrosis. Together, all stakeholders can help expand our understanding of the underlying pathophysiology of CF and advance our ability to bring new treatments to the benefit of individuals with CF.

Conflict of interest

The authors report no conflicts of interest related to the subject of this manuscript.

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